Brief Communication Communication brève

Toxocara vitulorum in a bison *(Bison bison)* herd from western Canada

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Abstract — The discovery of the parasite *Toxocara vitulorum* in bison calves in the province of Manitoba, Canada is discussed. This parasite is more commonly found in the small intestines of bovid calves living in tropical and subtropical regions of the world. This is the first time that *Toxocara vitulorum* has been reported from hosts in Canada.

Résumé — *Toxocara vitulorum* dans un troupeau de bisons *(Bison bison)* de l'Ouest canadien. La découverte du parasite *Toxocara vitulorum* chez des bisonneaux de la province du Manitoba, au Canada, est discutée. Ce parasite se trouve le plus fréquemment dans le petit intestin des veaux des bovidés vivant dans les régions tropicales et subtropicales du monde. C'est la première fois que *Toxocara vitulorum* a été signalé chez des hôtes au Canada.

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This brief communication reports *Toxocara vitulorum* infection in bison calves in the province of Manitoba, Canada. *Toxocara vitulorum* is a large roundworm commonly found in the small intestines of bovid calves living in tropical and subtropical regions of the world. Hosts most often infected are Asian water buffalo *(Bubalis bubalis)* and cattle *(Bos taurus, Bos indicus)*. This parasite has only infrequently been reported from more temperate areas of the world, including from cattle in Wales (~52°N) (1) and Australia (40°S) (2), and from North American bison *(Bison bison)* in Belgium (3). *Toxocara vitulorum* has a unique life cycle (Figure 1). Calves of host species tend to be the only age group from which the parasite's eggs are shed. These young animals ingest larvae found in the colostrum and milk from infected cows. The larvae mature in the small intestine of the calf and begin shedding eggs in the feces. It is also thought possible that calves might ingest embryonated eggs from the environment and after an extensive migration through the liver and lungs of the calf the larvae return to the small intestine, mature, and begin shedding eggs in the feces (4). Adult animals ingest embryonated infective eggs from the environment; the eggs hatch in the gastrointestinal tract and the released larvae migrate to somatic tissue in the host. Adult animals are generally thought to be free of mature adult worms and therefore lack any diagnostic evidence of infection such as eggs in feces. Larvae that have entered the somatic tissue assume dormancy or hypobiosis until, in adult host females, reproductive hormone levels near parturition cause activation and migration of the larvae to the mammary glands where they are shed through colostrum and milk. Males and non-breeding females appear to be dead-end hosts.

Although this parasite has been reported from bison in Belgium (3) and beef cattle in Florida (5), this is the first report from bison residing in North America. In 1992, Over et al (6) suggested that because new infections are acquired from ingestion of milk-borne larvae rather than infective eggs in the environment, transmission is more dependent on animal management than climate, meaning that whenever calves are permitted to suckle their mothers there is a chance for infections with *T. vitulorum* to occur regardless of how inhospitable the environment might be to infective forms such as embryonated eggs. If this is true, it may not be surprising that this brief communication reports *T. vitulorum* infection in bison calves in the province of Manitoba, Canada (~50°N), where weather conditions are much more severe than any other location previously reported. This is the first published account of this parasite in any species in northern North America but there are recent anecdotal reports of infected bison herds in Minnesota and North Carolina, USA that are unrelated to the present report.

On June 5, 2011 a veterinary practitioner in Manitoba was asked to perform a necropsy on a 3-week-old bison calf from a

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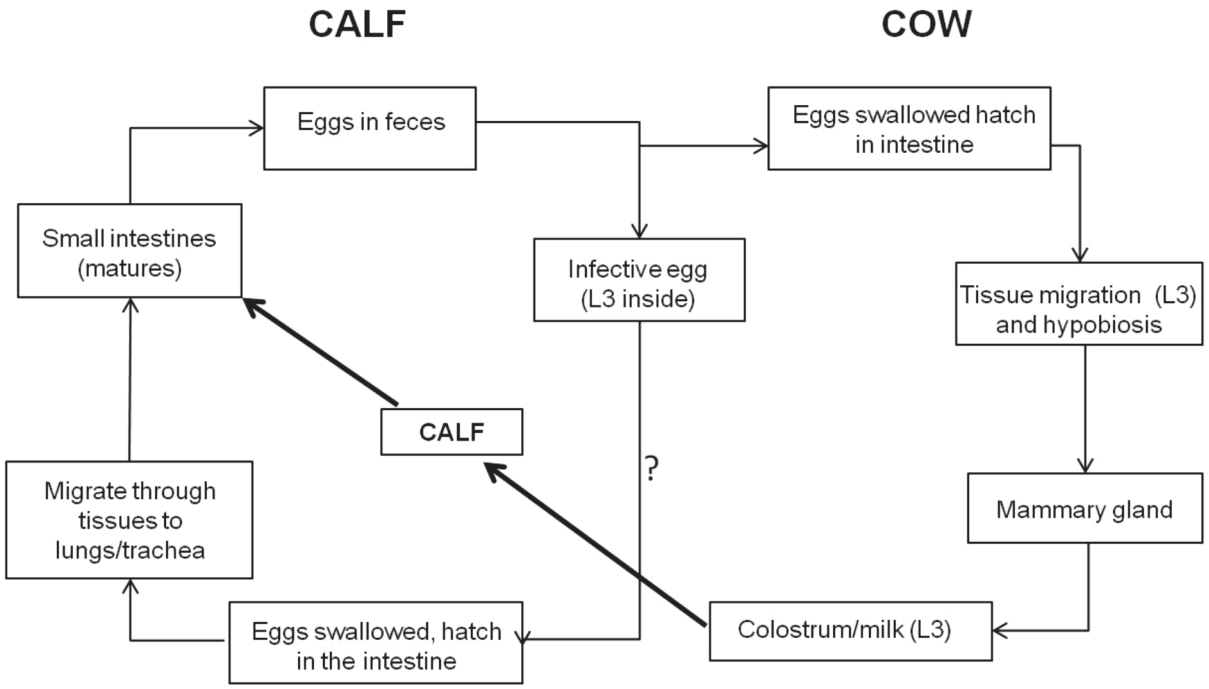

Figure 1. The life cycle of *Toxocara vitulorum* in Asian water buffalo *(Bubalis bubalis)*. With permission from Starke-Buzetti, 2006.

herd containing several "poor doing" calves of a similar age, 3 of which had died after showing diarrhea, anorexia, and dehydration. The small intestine (jejunum) was inflamed and congested with a large mass of adult nematodes occluding the lumen. The veterinarian tied off and removed this portion of the intestine and sent the specimen to the provincial veterinary laboratory in Winnipeg, Manitoba where it was determined by traditional morphologic examination of the parasites and associated eggs that they were probably *T. vitulorum* (Figure 2). Feces from 10 cows and 5 of the remaining 9 calves in the herd were subsequently sampled and examination by standard fecal flotation methods at the provincial veterinary laboratory in Winnipeg showed that the *T. vitulorum* infection was present in several calves. All calf samples had eggs consistent with *T. vitulorum* but samples from all cows were negative.

The intestine of the infected calf was forwarded to the parasitology research laboratory, Department of Veterinary Microbiology, Western College of Veterinary Medicine, Saskatoon, Saskatchewan for further study. Three adult worms found in the lumen had 3 distinct lips at the anterior end which are a characteristic of ascarid worms. The worms were otherwise in poor condition and further morphological identification was not possible. Intestinal contents were examined using a sugar flotation technique (7). Eggs recovered were consistent in morphology to *T. vitulorum* which are sub-spherical, rough-shelled and measure 69 to 95 μm by 60 to 77 μm (Figure 3).

The adult worm specimen sent to the parasitology laboratory at Saskatoon was identified by molecular techniques. The DNA was extracted from a 20-mg tissue sample of a single adult worm, using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, California, USA). Sequences corresponding to the ribosomal RNA gene internal transcribed spacer 1 (ITS-1) and ribosomal RNA gene internal transcribed spacer 2 (ITS-2) of *T. vitulorum* were amplified in separate conventional polymerase chain reactions (PCR) performed on 2 μL of genomic DNA in 50-μL reactions containing 2.5 U *Taq* DNA polymerase (Quanta Bio Sciences, Gaithersburg, Maryland, USA), 2.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris/HCl, pH 8.3, 250 μM each of dNTPs and 20 pmol each of primers NC5 (5′-GTA GGT GAA CCT GCG GAA GGA TCA TT-3′) and NC13R (5′-GCT GCG TTC TTC ATC GAT-3′) for ITS-1 and NC13 (5′-ATC GAT GAA GAA CGC AGC-3′) and NC2 (5′-TTA GTT TCT TTT CCT CCG CT-3′) for ITS-2 (1,8). Reactions were incubated at 94°C for 5 min followed by 40 cycles of (30 s at 94°C, 30 s at 60°C and 30 s at 72°C) and a final extension of 5 min at 72°C. Purified PCR products for ITS-1 and ITS-2 were sequenced with the respective amplification primers at the National Research Council Plant Biotechnology Institute, Saskatoon, Saskatchewan. Raw sequence data were processed and assembled using the Staden Package (9). Sequences have been deposited to GenBank (Accession numbers JQ083351 and JQ083352). Edited sequence data were compared to the NCBI GenBank nucleotide database using BLASTn (10) and were found to be 99% identical to previously published gene sequences for ITS-1 and ITS-2 of *T. vitulorum* over 500 and 443 bp, respectively. The sequences were clearly distinguishable from other *Toxocara* ITS sequences with $< 91\%$ sequence identity.

The parasitized animals are a breeding group of approximately 50 wood bison *(Bison bison athabascae)* that is segregated for most of the year from a larger breeding herd of about 400 plains bison *(Bison bison bison)*. There is also a 500-head bison feedlot

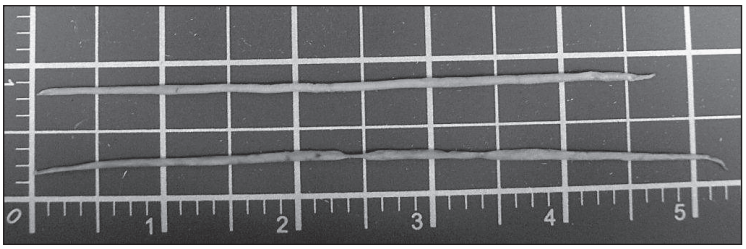

Figure 2. Adult *Toxocara vitulorum* worms taken from the upper small intestinal tract of a bison calf at necropsy. Numbers represent inches.

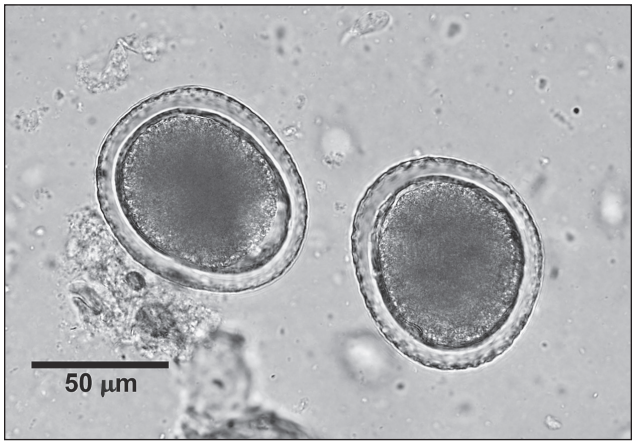

Figure 3. Unembryonated *Toxocara vitulorum* eggs found in the intestinal contents of a bison calf at necropsy.

on the property but animals leaving the breeding herd for the feedlot are never returned to the herd. The affected group is kept on tame grass pasture separate from the main plains bison herd for calving and the subsequent breeding season but is turned onto crop land to forage on corn for the winter months with the larger herd. Weaning and preventative health care procedures such as vaccination and parasite control are carried out when the animals are processed in late December or January. Anthelmintic used during processing consists of an application or injection of an avermectin type anthelmintic. Fenbendazole (Safeguard; Intervet; Merck Animal Health, Summit, New Jersey, USA) crumbles are fed on pasture at the recommended dose for cattle to animals that develop diarrhea or are doing poorly during the winter months.

The Manitoba bison farm has existed for approximately 15 y and over time has mostly been a closed operation. A number of years ago bulls purchased in northern USA were imported into the herd and most recently 11 wood bison heifers were purchased and added within the last 3 y from a herd in northern Alberta, Canada. Regular routine fecal parasite checks and egg counts have been performed in the Alberta adult herd without encountering ascarids but calves from that herd have not been routinely tested. The original source of the parasites remains unknown but if the parasite life cycle in bison is the same as in Asian water buffalo, the source of infection would likely be the replacement heifers. We are hoping to sample the next calf crop from the implicated Alberta herd to confirm or rule out this possibility.

The life cycle of *T. vitulorum* in bison is unknown but is unlikely to differ significantly from that in Asian water buffalo, in which transmission occurs after calves ingest larvae found in the colostrum and milk from infected cows (Figure 1). Adult animals are thought to be free of adult worms and therefore lack any diagnostic evidence of infection such as eggs in feces, which explains the lack of positive fecal tests in both the adults of the affected herd and the possible source herd in Alberta (4). However, similar to ascarid infections in carnivores, adult worms are sometimes passed in the feces of affected calves and might be noticed by observant livestock producers. This is the only worm in calves, other than tapeworms, that could be diagnosed this way.

Treatment of adult bison with anthelmintics is unlikely to be effective because of the inability of the drugs to affect hypobiotic larval forms (11). Treatment of young calves is appropriate and effective but administration of anthelmintics to bison calves on pasture is difficult if not impossible because of their nature and the probability of maternal intervention in the handling process. Bison mothers are very protective and there is a strong herd instinct to repel threats to herd members of any age. In general, the avermectins (12,13) are effective in both pour-on and injectable formulations against *T. vitulorum* in calves under 3 mo. The use of fenbendazole (5,14) has been very successful in young cattle but oral administration of an anthelmintic is unlikely in suckling bison.

The affected bison herd was essentially a closed herd except for the imports noted and had no known contacts with Asian water buffalo or cattle. Feed for the bison is produced on the farm. The source of the infection remains unknown, but there are plans for further examination of this and other herds in Manitoba through a fecal survey of calves under the age of 3 mo. It is possible that inapparent infections in other Canadian bison herds exist because, unless calves are clinically ill with diarrhea, fecal samples from bison calves are not routinely examined for parasites prior to weaning. CVJ

References

1. Jones JR, Mitchell ESE, Redman JS, Gilleard JS. *Toxocara vitulorum* infection in a cattle herd in the UK. Vet Rec 2009;164:171–172.
2. Roberts JA. *Toxocara vitulorum* in ruminants. Helminthological Abstracts 1993;62:151–174.
3. Goossens E, Dorny P, Vervaecke H, Roden C, Vercammen F, Vercruysse J. *Toxocara vitulorum* in American bison *(Bison bison)* calves. Vet Rec 2007; 160:556–557.
4. Starke-Buzetti WA. *Toxocara vitulorum* in livestock. In: Holland CV, Smith HV, eds. Toxocara: The Enigmatic Parasite. Cambridge, UK: CAB International, 2006:260–277.

5. Davila G, Irsik M, Greiner EC. *Toxocara vitulorum* in beef calves in North Central Florida. Vet Parasitol 2010;168:261–263.
6. Over HJ, Jansen J, van Olm PW. Distribution and impact of helminth diseases of livestock in developing countries. FAO Animal Production and Health Paper #96, FAO, Rome 1992:221 pp.
7. Foreyt WJ. Veterinary Parasitology Reference Manual. 5th ed. Ames, Iowa: Iowa State University Press, 2001:5.
8. Zhu X, Gasser RB, Jacobs DE, Hung GC, Chilton NB. Relationships among some ascaridoid nematodes based on ribosomal DNA sequence data. Parasitol Res 2000;86:738–744.
9. Staden R, Judge DP, Bonfield JK. Sequence assembly and finishing methods. Method Biocheml Anal 2001;43:303–322.
10. Altschul SF, Gish W, Miller W, Myers EW, Lipman EJ. Basic local alignment search tool. J Molec Biol 1990;215:403–410.
11. Roberts JA. Preventative treatment against toxocarosis in bovine calves. Vet Parasitol 1992;44:111–118.
12. Avcioglu H, Balkaya I. Efficacy of eprinomectin against *Toxocara vitulorum* in calves. Trop Anim Health Prod 2011;43:283–286.
13. Avcioglu H, Balkaya I. A comparison of the efficacy of subcutaneously administered ivermectin, doramectin, and moxidectin against naturally infected *Toxocara vitulorum* in calves. Trop An Health Prod 2011;43:1097–1099.
14. Mahieu M, Naves M. Incidence of *Toxocara vitulorum* in Creole calves of Guadeloupe. Trop An Health Prod 2008;40:243–248.